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fruitless, doublesex and the genetics of social behavior in *Drosophila melanogaster*

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Summary

Two genes coding for transcription factors, *fruitless* and *doublesex*, have been suggested to play important roles in the regulation of sexually dimorphic patterns of social behavior in *Drosophila melanogaster*. The generalization that *fruitless* specified the development of the nervous system and *doublesex* specified non-neural tissues culminated with claims that *fruitless* was both necessary and sufficient to establish sex-specific patterns of behavior. Several recent articles refute this notion, however, demonstrating that at a minimum, both *fruitless* and *doublesex* are involved in establishing sexually dimorphic features of neural circuitry and behavior in fruit flies.

Introduction

Modern biologists are accustomed to thinking in precise terms about how genes control anatomical and physiological traits, by encoding networks of regulatory developmental programs and/or cellular functions. Behavioral traits are inherently complex, and deciphering the connections between genotype and behavioral phenotypes requires understanding how genes control the development and physiological functions of the relevant neural systems. This article focuses on recent findings concerning two genes of *Drosophila melanogaster*, *fruitless (fru)* and *doublesex* (*dsx*), and their effects on the development of neural systems that generate two sexually dimorphic social behaviors, courtship and aggression. Both *fru* and *dsx* are part of the sex determination hierarchy (SDH) of genes in *Drosophila* that control both morphological and behavioral sexual dimorphisms. The two genes have distinct mutant phenotypes: *fru* mutations disrupt male sexual behavior, and *dsx* mutations scramble the normal morphological markers of males and females. These phenotypes suggested that *fru* and *dsx* function in distinct anatomical domains, i.e., that *fru* specifies sex-specific neural development while *dsx* specifies morphological sexual dimorphisms [1–4]. A few years ago, transgenic manipulations designed to reverse the normal sex-specific patterns of *fruitless* expression yielded phenotypes in which male flies behaved like females and females like males [5–7]. These results led to suggestions that *fru* alone was both necessary and sufficient for the generation of sex-specific patterns of behavior [5–8]. That notion has been challenged by more recent findings, and this review will examine evidence indicating that *fru* and *dsx* interact to

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specify many sexually dimorphic features of neural circuitry and behavior. Several comprehensive reviews relating to this topic have been published in recent years [9–11].

Sex-specific regulation of *fru* and *dsx* expression

Transcripts of both *fru* and *dsx* undergo sex-specific splicing that is regulated by upstream genes in the sex-determination hierarchy of *Drosophila*. The ratio of X-chromosomes to autosomes determines that splicing factors encoded by *Sex-lethal* (*Sxl*) and *transformer* (*tra*) genes are expressed only in females [12,13]. Tra and Tra-2, another splicing factor that is not sex-specifically expressed, in turn regulate female-specific splicing of *fru* and *dsx* transcripts. Both *dsx* and *fru* code for transcription factors, *dsx* for proteins of the zinc-finger family and *fru* for proteins resembling the BTB-zinc-finger family of transcription factors. Female-specific splicing of *dsx* transcript results in distinct male (Dsx^M) and female (Dsx^F) proteins [9,13,14]. The *fruitless* gene is more complex, with multiple promoters and alternative exons. Only RNA transcribed from the *fru*P1 promoter is subject to female-specific splicing by Tra and Tra-2 [15,16]. This female splice variant is not translated into protein, while default splicing of the *fru*P1 transcript in males generates Fru^M protein. In fact, three Fru^M isoforms with different zinc-finger domains can be produced in males by alternative splicing of 3′ exons [15,17].

The sex-specifically spliced *fru*P1 transcript is expressed in neural tissues in both males and females beginning in late third instar larvae, and peaking in 1–2-day old pupae. Generally similar patterns were labeled in the brains of male and female pupae by in situ hybridization, and there were some male-specific cell groups in thoracic and abdominal ganglia [15,18,19]. Fru^M protein was detected only in males, in the nuclei of about 1700 neurons in the pupal and adult CNS. The Fru^M-expressing neurons occur in 20 clusters or loosely aggregated groups that are widely distributed throughout the male brain, optic lobes, and ventral nerve cord [19]. The locations of many of these cell groups correspond with anatomical regions that had been implicated in the control of sexual behavior by earlier studies of genetic mosaics [20, 21]. Moreover, the effects of various *fru* alleles on either quantitative or qualitative features of the gene’s cellular expression patterns seem to correlate with the widely differing courtship phenotypes of different *fru* mutants [18,22], lending support to the hypothesis that Fru^M controls the development and/or the function of the neural circuitry for male sexual behavior [3].

An anti-Dsx antibody that detects both Dsx^M and Dsx^F labeled about 500 cells in the pupal CNS, with no evident anatomical sexual dimorphism. A marked sexual dimorphism was evident in adults, however, with much the same pattern of Dsx^M cells in pupal and adult males, and only a few cells labeled in adult females [23]. About half the Dsx-expressing neurons are in the abdominal ganglion, and another 80 are in thoracic ganglia. The brain contains two clusters of Dsx-expressing cells in the posterior protocerebrum as well as a few scattered neurons in the subesophageal ganglion [23]. Considering these results together with evidence for anomalous courtship behavior caused by *dsx* mutations [24,25], possible interactions of *fru* and *dsx* in specifying certain sex-specific behavioral patterns were suggested [23]. In fact, most *dsx*-expressing neurons in pro-and mesothoracic ganglia co-express Fru^M [26], and about half of the Dsx^M neurons in the brain and abdominal ganglion of male pupae co-express Fru^M [17,26]. Interactions between these two genes in the development of specific sexually dimorphic features of neural circuitry and behavior will be considered below.

Additional features of *fruitless* expression, beyond those indicated by Fru^M immunostaining, have been revealed by GFP reporters of several *fru* promoter-GAL4 drivers [6,27–29]. These *fru*-GAL4 drivers are expressed in similar patterns in the central nervous systems of males and females, although each apparently defines a different subset of the cell population revealed by

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Fru^{M} protein staining. Reporters of *fru*-GAL4 expression also revealed *fru* expression in many peripheral sensory structures, including olfactory, auditory, gustatory, and mechanosensory neurons in a variety of appendages [6,27,28], and much of this was confirmed by anti-Fru^{M} staining. Thus, thinking about *fruitless* function has expanded to include peripheral sensory neurons. Finally, some *fru*-GAL4 drivers have been used productively to characterize the structure and function of targeted cell groups [29–32], yielding important evidence for sexual dimorphisms in the numbers and neurite projections of some *fru*-expressing neurons, as described below.

Sexual dimorphisms in the structure of the adult fly CNS

Long before *fruitless* was suspected of specifying sex-specific neural circuits, three types of sexual dimorphisms in the structure of the adult fly CNS were documented by conventional neuroanatomical methods. These include male-female differences in (1) the size or volume of defined fiber tracts or neuropil regions, (2) the detailed morphology of identified neurons, and (3) patterns of neuroblast divisions, implying different numbers of particular types of neurons. Thus, neuroanatomists counted more mushroom body fibers in females than in males [33], and determined that certain brain neuropil areas were larger in adult males than in females [34]. Sexually dimorphic projections of sensory neurons into the prothoracic ganglion were visualized by cobalt fills of neurons innervating leg taste bristles [35]. Since the axons of females carrying *tra* or *Sxl* mutations were found to have male-specific branches, these gustatory axons provided the first clear evidence for a neuroanatomical dimorphism being controlled by genes of the SDH. Meanwhile, at the back end of the fly, a sexual dimorphism in the developing CNS was discovered by labeling dividing neuroblasts with bromodeoxyuridine [36]. Neuroblasts in the terminal abdominal ganglion that stopped dividing in females as third instar larvae continued to divide in males for another day, thereby producing about 20 extra neurons. Genetic analysis of this neuro-developmental dimorphism indicated that it is controlled by *tra, tra-2,* and *dsx,* thus providing evidence that cells intrinsic to the CNS are controlled by genes of the SDH.

Molecular genetic tools and manipulations of *fruitless* have revealed further examples of all three types of neuroanatomical sex differences, and further evidence that they are specified by the actions of genes of the SDH. Except for a few insights concerning the role of programmed cell death, however, we remain largely ignorant of the developmental mechanisms by which *fru* and *dsx* gene products generate these sexual dimorphisms.

Sexual dimorphisms in the olfactory system

Recent advances in understanding of the *Drosophila* olfactory system [37] include new evidence for neuroanatomical sexual dimorphisms. Sensory neurons in olfactory sensilla of the antennae send axons through the antennal nerve to terminate in glomeruli of the antennal lobe. While it has long been known that enlarged sex-specialized olfactory glomeruli exist in other insect species [38], clear evidence for sexually dimorphic antennal glomeruli in *Drosophila* is relatively recent [39]. The subset of olfactory receptor neurons in the antennae that express *fru*-GAL4 appear to project to a small number of sexually dimorphic glomeruli [6,8,28]. The volumes of these glomeruli were larger in males than in females of control genotypes, but not in genotypes engineered to prevent sex-specific splicing of *fru* transcripts, showing that sex-specific splicing of *fruitless* transcripts specifies the larger size of these glomeruli in male flies [28]. The possibility that synaptic contacts might be made between Fru^{M}-expressing sensory neurons and second order projection neurons that also express Fru^{M} was raised by the presence of Fru^{M}-expressing cell bodies near the antennal lobes [8, 28]. Such connections were elegantly demonstrated recently using a photoactivatable form of GFP to specifically label *fru*-GAL4-expressing neurons that innervate a single glomerulus

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[32]. With this approach, the processes of six projection neurons that innervate the sexually dimorphic DA1 glomerulus were traced to their terminal branches in the lateral horn of the protocerebrum. A small sex difference was detected in the fine structure of these arborizations, with a small male-specific ventral branch [32]. This sexually dimorphic pattern of axonal branching appears to be specified by Fru^{M}, since small ventral branches were observed in the DA1 projection neurons of females expressing Fru^{M}, but were not detected in *fru* mutant males [32].

Since the olfactory receptor neurons and the projection neurons that innervate the DA1 glomerulus respond to cis-vaccenyl acetate [40–43], a male anti-aphrodisiac compound transferred from male to female flies during copulation [44,47], it was suggested that this higher order morphological difference might account for behavioral differences in male and female responses to this pheromone [32]. That remains to be established, however, as many complexities exist in relation to the behavioral significance of cVA (it acts as an anti-aphrodisiac in males, but can serve as an attractant in both males and females [45]), in the receptors involved in detecting it [46,47], and in the physiological role of the circuitry described by these studies. Moreover, Fru^{M} is expressed in olfactory receptor neurons that project to other than sexually dimorphic glomeruli, and neurons expressing different olfactory receptors project to some of these glomeruli [48,49]. Nonetheless these results illustrate that Fru^{M}–expression can define at least the initial stages of a sensory system concerned with courtship behavior, and they suggest the possibility that Fru^{M}–expression might enable further tracing of neural circuitry that is concerned with sexual behavior.

Morphological differences between fru-expressing neurons in male and female brains

In the original mapping of cells in the CNS that express Fru^{M}, approximately 1700 neurons were classified into 20 groups [19]. This classification scheme has been refined recently by analyzing both cell body positions and trajectories of the primary neurites labeled by GFP reporters of fru^{NP21}-GAL4, which is expressed in 82% of the Fru^{M} staining neurons in male brains. As a result, the fru^{NP21}- expressing neurons in the brain have been subdivided into 48 identifiable clusters [30]. For most of these clusters, similar numbers of cells express fru^{NP21} in male and female adult brains, but two clusters are male-specific, and seven have significantly more cells in males than in females [30]. On average, in each brain hemisphere, fru^{NP21}-GFP labeled 480 neurons in males, and 336 neurons in females. Recognizing that these sexually dimorphic neuron numbers could arise from differences in either neuroblast proliferation or cell death, the MARCM method (Mosaic Analysis with a Repressible Cell Marker [50]) was used to ask whether female cells could be rescued by eliminating three cell-death genes, *hid*, *grim* and *rpr* [29,30]. Small clonally-derived groups of cells that were homozygous for a deletion that eliminated the cell-death genes were generated in females. In two of the sexually dimorphic cell groups, the mAL cluster just medial to the antennal lobes and the P1 cluster in the dorsal posterior brain, increased cell numbers were found in hid^{-} $grim^{-}$ rpr^{-}clones of adult females [29,30]. The results suggested that a cell-death program normally removes these *fru*-expressing neurons from female brains. Interestingly, genetic evidence suggests that the cell-death programs are regulated differently in these two cell clusters: that cell-death is inhibited in mAL cells of males by Fru^{M} [29], and that it is activated in P1 cells of females by Dsx^{F} [30].

Not only do males have a greater number of mAL cells than females (30 vs. 5 per hemisphere), there are clear sex-specific differences in the arborizations of these neurons [29]. In order to visualize the morphological details of individual mAL neurons, the MARCM method was used to limit the expression of the GFP reporter to small clonal groups of cells. The axonal processes of mAL neurons were similar in males and females, with varicose arborizations in the

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contralateral superior lateral protocerebrum. The dendritic branches, however, are sexually dimorphic. All five female mAL neurons have a single contralateral dendritic branch in the subesophageal ganglion, which is forked, whereas the larger male mAL cluster includes cells with exclusively contralateral dendrites and others with dendritic branches on both sides of the subesophageal ganglion, none of which were forked [29]. Analysis of the morphology of mAL neurons in *fru* mutant males revealed only female-like dendritic branches in most cells, suggesting that Fru^M specifies the development of the male-specific dendritic morphology [29].

Sexually dimorphic features of *fru*-expressing neurons in the abdominal ganglion

A distinctive group of large *fru*-expressing serotonergic neurons at the posterior tip of the abdominal ganglion (SAbg neurons) innervates male reproductive organs and is implicated in the transfer of sperm and seminal fluids during mating [22,51]. These neurons were the subject of a recent study that provided important evidence for transcriptional codes involving combinations of different Fru and Dsx isoforms in specifying neuronal identity. This study involved a new *fru* allele, $fru^{\Delta C}$, that contained a small deletion in the coding sequence of exon C, one of four alternative C-terminal exons that encode zinc-finger domains [17]. By combining the $fru^{\Delta C}$ allele and another *fru* allele that lacks all Fru^M isoforms with *fru*-GAL4 targeted expression of individual Fru^M isoforms, it was possible to evaluate the role of different isoforms in specifying neuronal phenotypes and aspects of sexual behavior. Thus, for the male specific Muscle of Lawrence (MOL) in the dorsal abdomen, whose appearance requires Fru^M expression in the motor neuron that innervates it [52], the Fru^{MC} isoform was found to be both necessary and sufficient for formation of the MOL and the associated motoneuron [17]. A more complex picture emerged for the male-specific serotonergic neurons in the abdominal ganglion, however. These cells were completely eliminated in Fru^M-null males, and greatly reduced in number in Fru^{MC}-null males, which still produce Fru^{MA} and Fru^{MB} isoforms [17]. Results of earlier studies using anti-5HT staining to try to label these cells in females suggested that Fru^M controls 5-HT expression rather than the presence or absence of the SAbg cells [51]. When Fru^M-immunostaining was used to count neurons in abdominal ganglia of Fru^{MC}-null males, however, the results suggested that a subset of the SAbg neurons failed to develop in Fru^{MC}-null mutants [17]. Moreover, efforts to rescue the SAbg cells by expressing different Fru^M isoforms in Fru^M-null males suggested that each Fru^M isoform rescued only a subset of the normal male complement of SAbg neurons. These results indicate that multiple Fru^M isoforms are required for the development of these cells. Furthermore, cell counts in different sex determination gene mutants suggested that both Fru^M and Dsx^M contribute to the formation of normal numbers of these male-specific serotonergic neurons [17]. These findings resonate with older evidence that *dsx* produces extra neurons in male abdominal ganglia by prolonging neuroblast proliferation [36]. These results clearly indicate that transcriptional codes involving different combinations of *fru* and *dsx* gene products specify different components of the neural circuitry concerned with sexually dimorphic patterns of behavior.

Is Fru^M sufficient for male behavior?

The hypothesis that Fru^M expression in pupal and adult nervous system is sufficient to generate male courtship behavior was tested by forcing expression of Fru^M in female flies. Two research groups used gene targeting by homologous recombination to generate engineered *fru* alleles that forced either male- or female-specific splicing of the *fru*P1 transcript. One team directly engineered sites required for sex-specific splicing of *fru* P1 transcripts such that fru^M and $fru^{\Delta tra}$ alleles encode transcripts that are always spliced into the male form encoding Fru^M, and a fru^F allele prevents male-specific splicing [5]. The other group produced a *fru*P1-GAL4 driver by recombining the GAL4 coding sequence into *fru*, then used it to drive expression of

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UAS-RNAi transgenes that disrupted sex-specific regulation of the endogenous *fru*P1 transcripts [6]. Female flies that expressed Fru^M by either of these strategies performed some distinctly male patterns of courtship towards wild-type virgin females, providing evidence of the sufficiency of Fru^M to specify the neural circuitry responsible for some male-specific behavioral patterns. Many features of courtship behavior in these masculinized females, however, were different from that of normal males. Females with their *fru*P1-cells masculinized by expressing $tra2^{IR}$ performed early steps of courtship, but not later stages (wing extension, licking, and copulation attempts) [6]. The fru^M and $fru^{\Delta tra}$ females reportedly performed all steps of courtship except for attempting to copulate, except that data in this paper showed that licking was significantly subnormal in these masculinized females [5]. Furthermore, in a later study, audio recordings of fru^M and $fru^{\Delta tra}$ females revealed that their courtship songs were abnormal and much less frequent than those of control males [26]. Since *dsx* mutations also produce abnormal song phenotypes [25], and most Fru^M-expressing neurons in the mesothoracic segment of the CNS co-express Dsx^M [26], it is likely that both genes contribute to specifying the neural systems for song. Thus, in spite of claims to the contrary, Fru^M expression in females is not sufficient to specify normal male courtship behavior.

Aggression is a second sexually dimorphic behavior in *Drosophila* that is influenced by *fruitless* [7]. Both male and female flies display patterns of aggression in same sex pairings, and many of the behavioral patterns seen are sex-specific [53]. By using the fru^F and fru^M alleles described above [5], it was demonstrated in same sex pairings that fru^F males displayed female patterns of aggression and fru^M females displayed male patterns [7]. Similar results were obtained when male nervous systems were feminized or female nervous systems were masculinized by pan-neural expression of either UAS-tra^F or UAS-tra^{IR}, respectively [54], except that the tra^{IR}-expressing females did not perform male-type wing threats. The conclusion of these studies too, was that Fru^M expression was necessary and sufficient to transform patterns of aggressive behavior from female-like to male-like. Note, however, that forcing both Fru^M and Dsx^M expression in female nervous systems (with tra^{IR}) was not sufficient to produce male-like wing-threat displays.

Behavioral functions of subsets of *fru*-expressing neurons near the brain midline

In an attempt to identify neurons responsible for sex-specific patterns of aggression, a collection of 60 enhancer trap-GAL4 drivers was screened for the ability to induce sex-reversed behavioral patterns when used to manipulate *transformer* expression [54]. Five of these GAL4 drivers produced partially masculinized (GAL4-tra^{IR}) females that performed male courtship behavior, and two of these genotypes also displayed male patterns of aggression. The brains of these five types of GAL4-tra^{IR} females were stained with anti- Fru^M to determine whether Fru^M expression in any particular cluster(s) might be correlated with the female's ability to fight like males. While there was no single cluster where Fru^M-staining correlated absolutely with male-like fighting patterns, four clusters were identified where the numbers of Fru^M–staining cells in GAL4-tra^{IR} females that fought like males were comparable to those in normal males, and where Fru^M–staining was consistently weaker in GAL4-tra^{IR} females that fought like females [54]. Three of these four clusters are close to the midline in the brain (*fru*-mAL, *fru*-aSP2, *fru*-mcAL), and one is in the ventral nerve cord (*fru*-PrMs). They include the mAL cluster that has sexually dimorphic cell numbers and arborizations [29] and also was selectively missing from the Fru^M-staining pattern in fru^1 mutant males that show high levels of male-male courtship [22].

Understanding of the structure and function of specific subsets of neurons requires manipulations that target identifiable cells or clusters. In a recent attempt to localize the behavioral functions of *fru* to a particular cell cluster, the MARCM method was used to

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generate small clonal groups of *fru*^{NP21}-GAL4 –expressing cells that were both labeled by GFP and homozygous for *tra*^{1}, thereby masculinizing the cells [30]. By assaying courtship behavior and evaluating the spatial patterns of GFP/*tra*^{1} neurons in 205 MARCM females, this study was designed to determine whether masculinization of any *fru*^{NP21}-expressing cells or clusters correlated with the ability of the MARCM females to court like males. Only 16 of the 205 mosaic females displayed early steps of courtship behavior, and none performed advanced steps or sustained bouts of courtship [30]. That may not be surprising since most of their neural circuitry was still female. As above, in no single cell cluster did the *tra*^{1} genotype correlate perfectly with male-like behavior. The best correlation between genotype and behavior was found for the P1 cluster, a group that is normally found to express *fru*^{NP21} only in males. This cluster was masculinized in 13 of the 16 MARCM females that performed any courtship, and in every one of the 10 most active courters [30]. The P1 cells were not masculinized in three weak courters, however, thus one cannot conclude that Fru^{M}/Dsx^{M} expression of these cells is absolutely necessary for weak displays of courtship. Nor are members of this cluster sufficient for male courtship, since P1 cells also were masculinized in 12 of the 189 MARCM females that displayed no courtship in these behavioral assays [30]. Given the complexity of the behavioral phenotype and the numbers and diversity of Fru^{M}-expressing neurons, it may not be reasonable to expect that masculinization of any one cell cluster will prove to be both necessary and sufficient to initiate courtship in an otherwise female brain. It is fair to say that the genetic sex of cells in the P1 cluster has an important influence on a fly's ability to perform early steps of male courtship behavior. A note of caution, however, is that early steps in aggression in both males and females involve approach and tapping behaviors that may be difficult to distinguish from early steps of courtship behavior.

Finally, it may be significant that these first attempts to relate the genetic sex of subgroups of Fru^{M}-expressing neurons with behavioral patterns of courtship and aggression have all implicated neurons that lie within Fru^{M}-clusters close to the brain midline [22,30,54].

Conclusions

Behavioral traits are more complex than neuroanatomical ones, and can be difficult to measure reliably. This is especially true for social behaviors that are influenced by multiple behavioral and sensory interactions with another fly. Male courtship behavior, for example, has proven to be highly sensitive to assay conditions [55]. In addition, general statements of necessity and sufficiency for genes that regulate behavioral phenotypes often need to be revised and qualified in light of new evidence. While this may be disconcerting for *Drosophila* geneticists, who are accustomed to dealing with Mendelian traits, it is less surprising from the perspective of human behavioral genetics, where it has long been recognized that Mendelian behavioral phenotypes are the exception rather than the rule.

While expression of Fru^{M} proteins is necessary to specify the neural systems that generate male patterns of behavior, Fru^{M} alone is not sufficient to generate normal male patterns of behavior. At a minimum, *dsx* expression also is required and other genes may be involved as well [56]. Yamamoto [10,30] has suggested that three categories of neurons might be involved in establishing the circuitry underlying sexually dimorphic patterns of behavior: sex specific neurons requiring *dsx* and *fru* [17,26]; sex specific neurons requiring either *dsx* or *fru* [17, 57]; and non-sex specific neurons requiring neither gene [31,47]. Much of evidence described in this review supports this view.

Much remains to be done, however, to unravel the roles of genes in establishing sexually dimorphic patterns of behavior in fruit flies. At the genetic level, only a start has been made on deciphering the transcriptional codes that specify neuronal fate within sexually dimorphic circuitry [17,26,30]. It will be important to determine which of the three Fru^{M} isoforms are

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required to specify the phenotypes of identified subsets of Fru^M -expressing neurons, and how Dsx^M and Dsx^F influence neuronal numbers and phenotypes. It also will be important to identify genes that are directly regulated by Fru or Dsx proteins in the developing nervous system [58]. Current evidence suggests that Dsx proteins will regulate genes that control neuroblast proliferation and programmed cell-death, and that Fru^M isoforms will potentially regulate a broader array of targets, encompassing processes that specify both neuronal numbers and cellular phenotypes. At the neural systems level, important beginnings have been made on mapping the arbors of and connectivity between neurons within sexually dimorphic circuits [29,32], but far greater detail is required in the examination of these neurons, many more neurons must be examined, and the patterns of connectivity must be established at both light and electron microscopic levels. At the cell biological level, neurotransmitter and neurohormone identification and receptor, second messenger and ion channel distribution among neurons within Fru^M-associated circuitry has been lacking [22,59]. Information about these essential signaling functions will be needed to understand how sex-related neural systems functions. Although many questions remain concerning how sex-specific patterns of behavior get established and maintained in even the relatively simple fruit fly nervous system, with an ever-expanding array of powerful genetic and physiological methods available to address such questions, progress is likely to continue at a rapid rate.

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